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(54) Title: CHEMICAL SUPPLEMENTATION OF BONE

(57) Abstract

The present invention provides bone or bone tissue supplemented with at least a therapeutically useful compound, wherein said compound is concentrated within the bone matrix. The invention also provides a method of supplementing bone and bone tissue.

Chemical Supplementation of Bone.

The present invention provides bone and bone tissue supplemented with at least a therapeutically useful compound and in particular relates to a method for supplementing bone and or bone tissue with said compound(s).

Major allograft surgery has provided a solution to many reconstructive problems in musculoskeletal and maxillofacial surgery. The use of such surgery remains however, retarded by the frequency of infections that are often a disabling complication of such surgery. While the use of small frozen allografts has a very low rate of infection, major allografts have infection rates between 5 and 13%. This susceptibility to infection is probably multifactorial, with avascularity and antigenicity of the implanted graft contributing as well as the frequent extensive soft tissue excision, and potential for wound breakdown.

The use of allograft bone in orthopaedic practice is now well established both as morsellised and site specific structural grafts. The risk of infection, however remains a major complicating factor with such surgery. Like other forms of allograft surgery, the frequency of infection with allograft bone varies between 5 and 13.3%. The outcome in patients who develop infection is poor and often requires either two stage revision or amputation.

Infections typically arise early after allograft surgery with 75% of cases presenting within 4 months. Perioperative introduction of organisms is the presumptive mode of infection in the majority of these cases. The most common organisms isolated are gram positive (54%) followed by gram negative (36%) and mixed (10%).

Numerous attempts have been made to lessen the rate of infection in allograft surgery, particularly in the field of maxillofacial surgery. Perioperative antibiotic regimes are often employed, involving prolonged administration of antibiotics for up to 3 months, although no controlled studies have been performed to show the efficacy of these regimes. The theoretical problem of systemic antibiotic

administration in allograft surgery, particularly when using allograft bone is that the allografts are initially avascular and the antibiotics do not reach their target.

Attempts have been made to load allograft bone with antibiotics. In one such study morsellised graft was mixed with antibiotic solutions. More recently antibiotic supplemented bone allograft has been developed and used in the area of avulsive defects of the oral and maxillofacial skeleton. This technique employs demineralised particulate allograft bone and mixes it with purified gelatine powder and cephalothin and tobramycin. A canine model to test this preparation has shown a probable protection from post-operative infection when compared with conventional allografts.

Although these methods have been shown to display a decreased complication rate, the problem of infection in major allograft bone surgery is still a major concern. Furthermore, present methods of preparing allograft bone against infection require a large amount of preparatory work, are typically unsuitable where large bone grafts are required and depending on the methods used may not result in a product that has the same structural integrity as allograft bone. Thus, the problem of infection in major allograft bone surgery is largely unsolved and has severe consequences to patients who develop complications.

The present invention seeks to provide an improved bone and or bone tissue supplemented with at least a therapeutically useful compound. Moreover, the invention seeks to provide a simple and effective procedure for supplementing bone and or bone tissue with at least a therapeutically effective compound.

Throughout the specification, unless the context requires otherwise, the word "comprise" or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

For the purposes of the present invention the phrase "bone and bone tissue" encompasses bone substitutes which comprise any biological or synthetic material used to substitute for bone during reconstruction including materiall

- 3 -

processed from xenographt sources and chemicals manufactured for bone substitute purposes such as calcium phosphate and hydroxy appatite.

The present invention consists in a bone or bone tissue supplemented with at least a therapeutically useful compound, wherein said compound is concentrated within the bone matrix.

Unlike prior art products the present invention does not rely upon the use of binders, protective agents, gelatinisation agents or the like to associate therapeutic compounds with allograft bone or tissue. Rather, therapeutically effective compounds are delivered to and concentrated within the bone matrix by a process of iontophoresis. Preferably the concentration of the therapeutically effective compound within the bone is greater than the amount of therapeutically effective compound that might be absorbed into bone as a result of simple diffusion. It will be appreciated that the relative amount of therapeutic compound which might be loaded into any particular piece of bone will depend on (a) the safe *in situ* usage limits for that therapeutic compound, (b) the characteristics of the bone or bone tissue, (c) the biochemical characteristics of the particular compound selected and (d) the particular purpose for which the bone or bone tissue is being used.

Therapeutically effective compounds that might be employed in the invention include, but are not limited to: antibiotics, antifungal compounds and chemotherapeutic compounds, tissue growth factors (for example bone morphogenic protein), non-steroidal anti-inflammatory agents, such as indomethacin, neuromuscular agents affecting calcium and bone metabolism (such as calcitonin), anti-viral agents, anti-tuberculosis agents (such as rifampicin), anthelmintic agents (such as mebendazole), antiseptic agents, vitamins and minerals. Most preferably, the compounds that are loaded into the bone are compounds that form a salt in solution and ionise to a single positive or negative ion. Those of ordinary skill in the art will know such compounds.

- 4 -

If, for example, antibiotics are to be loaded into the bone or bone tissue the antibiotic compound is preferably selected from the following: flucloxacillin, gentamicin, cephalothin, ticarcillin, ciprofloxacin, nenzl-peniccillin, cefoperazone, cefuroxime, cephazolin and tobramycin. Most preferably the antibiotic is either flucloxacillin or gentamicin. When loaded into bone these compounds are preferably present at a concentration of between the minimum inhibitory concentration of the antibiotic and the concentration that would provide a total amount of antibiotic equal to the safe maximum single dose for systemic administration. For example, the maximum dose of gentamicin that might be loaded into allograft bone is about 200 mg/kg while the maximum dose of flucloxacillin is about 80 mg/kg.

If the therapeutically effective compound is an antifungal compound, the antifungal compound is preferably selected from the following: miconazole, and ketaconazole. When loaded into bone, these compounds are preferably present at a concentration of between the minimum inhibitory concentration of the antifungal and the concentration that would provide a total amount of antifungal equal to the safe maximum single dose for systemic administration.

If the therapeutically effective compound is a chemotherapeutic compound the chemotherapeutic is preferably selected from the following: 5-fluoro-uracil and vinblastin. Most preferably the chemotherapeutic is 5-fluoro-uracil.

In an alternative form, the present invention consists of a method for supplementing bone or bone tissue with a therapeutically effective compound, wherein said method employs the steps of:

(i) Exposing bone or bone tissue to a therapeutically effective compound; and

(ii) Applying a potential difference across said bone or bone tissue such that the therapeutically effective compound is concentrated within the bone or bone tissue.

Preferably, the therapeutically effective compound employed in the method is concentrated within the bone or bone tissue using an externally applied potential difference. Any externally applied potential difference may be used in the method, provided that it does not destroy the structural integrity of the bone or bone tissue. The potential difference that is used will depend on: (a) the thickness of the bone, (b) the time available to deliver the compound to the bone, (c) the compound which is to be loaded into the bone and (d) the temperature of the bone. Preferably the temperature of the bone during the loading process should be maintained below about 37°C.

If high externally applied potential differences are being used to load the bone with a therapeutically effective compound, then the method should be carried out in the presence of a means which is capable of cooling the bone or bone tissue. For example the method might be carried out in a refrigerated environment or alternatively might be carried out in a water bath.

It will be appreciated that the present invention is not limited to the loading of sectioned allograft bone. It might also be used *in situ* to deliver compounds into bone to treat medical disorders such as bone tumours. In such circumstances the therapeutically effective compound is preferable introduced at medically safe levels into the tissue surrounding the bone. A externally applied potential difference is then applied across the bone for sufficient time to concentrate the therapeutically effective compound within the bone. Preferably, the externally applied potential difference is selected such that it is capable of drawing and concentrating the therapeutically effective compound into the bone but does not effect the structural integrity of the surrounding tissue.

Therapeutically effective compounds suitable for use in the method are those which are capable of forming a soluble salt in solution. Preferably the compounds selected are capable of ionising in the presence of an externally applied potential difference to form either positive or negative ions. Examples of suitable compounds are described above. Most preferably antibiotics such as

- 6 -

flucloxacillin and gentamicin are used as the therapeutically effective compounds.

The concentration of therapeutically effective compounds that may be loaded into the bone or bone tissue will depend largely on the properties of the compound used and the time over which the compound is required to have a therapeutic effect. Preferably the compound is concentrated within the bone to a level which exceeds the amount of compound that might be diffused into the bone as a result of diffusion over an equivalent period of time. That is, when both the present method and a diffusion method are carried out over an equivalent period of time.

Applying an external potential difference across bone or bone tissue requires the use of at least two electrodes, one being located on one side of the bone and the other being suitable positioned on the other side of the bone. To ensure electrical contact between these electrodes they are each preferable surrounded by a medium capable of conducting electrical current. Preferably there are a plurality of electrodes on either side of the bone. While any electrode might be used in the method, the preferred electrodes are those that do not produce a chemical residue that would damage the bone on bone tissue. Suitable electrodes for use in the invention include, but are not limited to carbon, platinum, titanium, gold, noble metals, stainless steel, conductive plastic and the like.

In a highly preferred form of the invention the method is applied to allograft bone to load the bone with suitable therapeutic compounds prior to or during allograft surgery. Preferably the method is performed under aseptic conditions.

According to a particularly preferred form of the method, the section of bone to be treated is prepared and defrosted. It is then cut (in a manner which would be well known to those in skilled in the art) to an appropriate length with a slight excess at each end. Preferable that excess is in the order of about 1 to 10 mm.

To one end of the bone, a disc of an appropriate size to completely seal the medullary canal at that end is sealingly engaged to the bone. The disc can be made of any insulating material, such as acrylic, plastic etc. Sealing engagement between the bone and the disc may be achieved using, for example, a glue which is capable of bonding the disc to the bone, such as cyanoacrylate, and which is capable of being sterilised. Those of ordinary skill in the art will know such glues.

To the opposite end of the bone, a tube of an appropriate length is cemented to the bone, such that the tube sealingly engages onto the end the medullary canal (see Figure 1). The tube can be made of any insulating material, such as acrylic. The specimen is then placed in a beaker and immersed in a buffer solution such that the open end of the extended medullary canal is just above the level of the ionic solution. The buffer solution can be any solution capable of conducting electrical current, such as normal saline.

The medullary canal is then filled with an ionic solution of the compound to be loaded into the bone. Desirably the solution is sterile and consists of an antibiotic in ionic form such as gentamicin or flucloxacillin, but may also consist of antifungal compounds, chemotherapeutic agents or any other compound that ionises to a single ionic species in solution. The pH of solution should then be optimised to ensure maximal ionisation of compound.

Electrodes are then placed in the apparatus, with at least one placed vertically in the ionic solution, and a plurality vertical, equally spaced electrodes fixed to the side of the beaker, immersed in the buffer solution. The surrounding electrodes should then be connected electrically such that they act as one electrode. The electrodes can be made of any inert material such as carbon, platinum.

A potential difference is then applied across the electrodes until loading of the therapeutically effective compound into the bone is complete. The time required for the compound to be transferred in to the bone tissue will depend on (a) the

- 8 -

voltage - with increasing voltage the shorter the time period is required for transfer of the compound, (b) the thickness of the bone (c) the ionic compound used. Typically the maximum voltage that might be used in the method without the assistance of a suitable cooling means would be in the order of 100V, although there is theoretically no upper or lower limit to the voltage that can be used when a cooling means is employed. Desirably the temperature of the bone should not reach or exceed 37°C, at which temperature the collagen component of bone begins to degrade.

Voltages and times for application may be determined experimentally as described in the following examples.

The present invention is further described by the following non-limiting Figures and Examples:

Figure 1 illustrates a representative apparatus for transverse iontophoresis.

Figure 2 illustrates a representative apparatus for longitudinal or circumferential iontophoresis.

Figure 3 illustrates a apparatus for iontophoresis of tubular bone specimens.

Figure 4 illustrates a graph of gentamicin iontophoresis using sheep tibia.

Figure 5 illustrates a graph of flucloxacillin iontophoresis using sheep tibia.

Figure 6 illustrates a graph of gentamicin iontophoresis using human allograft.

Figure 7 illustrates a graph of flucloxacillin iontophoresis using human allograft.

Figure 8 illustrates a graph of the combined Iontophoresis using gentamicin and flucloxacillin.

Figure 9 illustrates a graph of hourly gentamicin washout per mg antibiotic available.

Figure 10 illustrates a graph of hourly flucloxacillin washout per mg antibiotic available.

Figure 11(a) illustrates gentamicin bioactivity against *Staphylococcus Aureus*.

Figure 11(b) illustrates flucloxacillin bioactivity against *Staphlococcus Aureus*.

Figure 12 illustrates a graph of a temperature plot.

Further features of the present invention are more fully described in the following Examples. It is to be understood, however, that this detailed description is included solely for the purposes of exemplifying the invention, and should not be understood in any way as a restriction on the broad description as set out above.

Examples

Example 1

Experimental work was carried out using mature Merino Sheep tibiae accessed from 'butcher's shops', and later using Human allograft bone which was rejected for implantation by the Perth Bone and Tissue Bank. The ionic compound used initially was methylene blue in 1% solution which forms positive ions and migrates away from the anode when a potential difference is applied. flucloxacillin and gentamicin were the antibiotics selected as the antibiotics for further study. Methylene blue was chosen for the initial work because its ease of visual identification obviated the need for assay techniques. It has a

molecular weight of 373 which is not very different from that of flucloxacillin (453) and gentamicin (460). This similarity means that it provides a good model to study the likely behaviour patterns of flucloxacillin and gentamicin ions using iontophoresis. Gentamicin exists in solution as positive ions of the sulphate salt, and flucloxacillin as negative ions and ionic aggregates of its sodium salt.

Preliminary work was done using $1cm^2$ discs or squares of cortical bone and was used to establish whether iontophoresis was effective in facilitating the movement of methylene blue ions in bone. In addition the technical aspects of how the technique's efficacy could be maximised were studied with particular reference to: The direction in which methylene blue is moved easiest under the influence of a potential difference through cortical bone; The effect of a pulsed field as compared with a unipolar potential gradient on the movement of ions; and the effects of varying the potential difference on the rate of movement of ions across the bone.

Pieces of bone were imbedded in epoxy cement, as an electrical insulator, leaving 2 surfaces free to apply the iontophoretic gradient across. The specimens were then sectioned and the penetration of the methylene blue measured. The apparatus used is shown in Fig1 & 2.

These served to study the feasibility of iontophoresis in bone, and the most effective method of applying the technique. Control experiments where methylene blue was applied to one surface of the cortical bone disc revealed very minimal penetration of the blue colour even after 20 minutes. In cases where a potential difference was applied the methylene blue was observed to migrate through the cortices.

The rate of facilitated diffusion was proportional to the potential difference applied and at 100 volts across a 4mm cortex, full penetration could be expected at approximately 4 minutes. There was no advantage apparent from using a pulsed field, as polarisation of the electrodes with a consequent fall in the current being passed appears to occur at about the time of full cortical

penetration, by the ions under investigation, and is therefore of little consequence. The technique was most effective in moving ions transversely across the cortex. It was less effective at moving ions circumferentially around a cortex or longitudinally in the direction of the haversian canals.

Example 2

Tubular Bone Allograft Model:

From this initial work a system to fully investigate the hypothesis was developed with more clinical relevance; Sections 2cm long were cut from the diaphysial region of the sheep tibia. They were sealed at one end by cementing on an acrylic disc using cyanoacrylate glue. The open end of the tube of bone had a 3 cm long acrylic tube cemented to it, effectively extending the medullary canal and thereby increasing its volume. The specimen was then placed in a beaker to the inside of which 3 vertical equally spaced carbon electrodes were fixed. The beaker was then filled with normal saline solution to just below the open end of the acrylic tube. Two ml of the ionic compound under investigation was placed in the medullary canal of the tibia and a carbon electrode lowered into it. The effect of a potential difference applied across the cortex could thus be studied. Control specimens were prepared in an identical fashion, except no potential difference was applied across the electrodes. Study specimens had the potential difference applied for 1 minute, 2 minutes, 5 minutes or 10 minutes.

All specimens were then removed and the medullary canal washed clean and dried. Axial sections were then cut using a diamond saw and the amount of penetration of the methylene blue measured. In addition histological sections were prepared 300μm thick to assess the penetration at a microscopic level. The methylene blue iontophoresis provided a qualitative analysis of iontophoresis in bone.

With the system set up as described, and methylene blue as the ionic solution the typical current that flowed during iontophoresis of the specimens was 40

- 12 -

mamp.   The blue coloration of the periosteal surface was noted by approximately 1.5 minutes and by 5 minutes was maximal.

Macroscopic evaluation of the specimens subsequently was done to evaluate the penetration of the methylene blue and microscopic evaluation done to assess the uniformity of penetration. The results of this are shown in Table 1

This work was duplicated using sections of human allograft tibia. The results were found to be comparable to the sheep model. The only appreciable difference between the two was that the sheep tibiae consisted purely of compact cortical bone whereas the human tibiae comprised cortical bone on the periosteal aspect, with cancellous bone on the endosteal aspect. This cancellous bone was no barrier to diffusion of the methylene blue. The rate of facilitated diffusion and the uniformity of penetration were comparable.

	Macroscopic	Microscopic
Control	Minimal Penetration (Staining of endosteal Surface with few Volkman's canals staining for variable distance)	Staining on endosteal surface with volkman's canals filled for variable distance. No staining of bony matrix
1 Minute	Penetration 1.5 mm	Uniform staining of matrix to level of penetration. Filling of Volkman's canals through full thickness of cortex
2 Minutes	Penetration 3 mm	Uniform staining of matrix to level of penetration. Filling of Volkman's canals through full thickness of cortex
5 Minutes	Penetration full thickness of cortex.(Patchy staining of periosteal surface)	Complete uniform penetration of entire thickness of cortical bone matrix and Volkman's canals

Table 1

- 14 -

Example 3

Quantitative analysis was then carried out to test iontophoresis as a means of antibiotic delivery in allograft bone. Specimens of sheep tibia were prepared as described above. The medullary canal was filled with 2 ml of 1 % gentamicin Sulphate solution (Delta West, Western Australia) or 1% flucloxacillin Sodium solution (Alphapharm) in distilled water. A potential difference of 100 volts was applied for 1 minute, 5 minutes or 10 minutes with controls being set up the same way for 10 minutes except no potential difference was applied. A recording of current passed for each specimen was made.

Each sample was then washed in water and dried. Samples from 10 the endosteal and periosteal surfaces were then taken by drilling the bone using a 3mm low speed drill, in 10 positions on each surface, and crushed to a fine powder. To a known mass of each sample was added distilled water (2ml) and the sample agitated using ultrasound. The sample was then centrifuged and the supernatant removed and the process repeated a further 2 times. The last wash was left to soak for 12 hours before centrifuging. The Fucloxacillin specimens were refrigerated to 4°C during this to minimise degradation by hydrolysis. The supernatant solutions were then analysed for antibiotic content; for gentamicin using Fluorescence Polarisation Immuno Assay (Abbot Axsym Analyser); and for flucloxacillin the assays were performed using High Performance Liquid Chromatography (HPLC) technique. From the levels in the supernatant solutions, the antibiotic concentrations in the bone samples were calculated.

To study the thermal effects of iontophoresis on bone, some samples were prepared and a fibre optic thermal fluorescence probe was introduced into the centre of the cortical bone via a 0.8mm drill hole in the long axis of the specimen. The apparatus was otherwise set up as above and then cooled to 10°C. The iontophoretic potential difference was then applied at 100 volts for 15 minutes and a continuous recording of the temperature made.

Initial work was carried out using the sheep tibia model of allograft tubular bone. For each control and iontophoretic group, 5 specimens were used. Samples from the endosteal and periosteal surfaces were processed separately. The results are shown in Fig 4 & 5.

It can be seen from these graphs, that both flucloxacillin and gentamicin ions are moved through sheep cortex by the iontophoretic gradient. The levels of gentamicin achieved in both endosteal and periosteal specimens plateau at about 150 mg/Kg. This compares with recommended peak serum concentrations after intravenous administration of the drug of 10 mg/L. The minimum inhibitory concentration of gentamicin to *Staphylococcus aureus* is approximately 0.25 mg/L (although this varies with different phage types). The peak in the endosteal specimens was reached by 1 minute, and the periosteal samples at 5 minutes. This is due to the fact that the ions have further to travel to reach the periosteal surface, and consequently take longer.

This work was supplemented by repeating the study using human allograft tibia. The scarcity of available allograft bone necessitated that each group contained only one specimen. All 4 specimens were contiguous 1cm sections of the diaphysis of the same non-irradiated tibia for each of gentamicin and flucloxacillin. The results are shown in figures 6 & 7.

Gentamicin and flucloxacillin behave in a similar fashion in human allograft bone to sheep bone. Again therapeutic levels of antibiotic are achieved although the time to reach maximal levels at all depths was 10 minutes. This increase in time probably reflects the increase thickness of the human specimens compared with the sheep specimens.

As gentamicin migrates from the anode and flucloxacillin migrates from the cathode, the next step to investigate was the possibility of simultaneous iontophoresis of each drug in opposite directions across sheep tibia specimens. The set up was as previously used, the gentamicin 1% solution being placed in the medullary canal with the anode and the flucloxacillin 1% solution being

placed in the beaker, thus bathing the cathode. The results of this are shown in Figure 8.

As can be seen here, gentamicin penetrates the full thickness of the cortex after 5 minutes of Iontophoresis, whereas the flucloxacillin levels in the periosteal samples only are elevated. The total antibiotic concentration is markedly elevated when compared with the controls.

Example 4

The bioavailability of the antibiotics was assessed by creating specimens using the above iontophoretic technique and applying the potential difference for 5 minutes. Samples were then drilled from the cortices and prepared as above for assay of the starting antibiotic concentration. Each specimen was then immersed in 20 mls of Normal Saline. This solution was poured off at intervals and antibiotic levels assayed in the solution. Each sample was immediately re immersed in fresh solution. In this way the rate of elution of the iontophoresed antibiotic could be studied. Again, the gentamicin samples were kept at ambient temperature the flucloxacillin samples being refrigerated to 4°C. The final elution assay was carried out at 14 days. The bioactivity of the antibiotics loaded into bone by iontophoresis was investigated. Specimens of sheep tibia were prepared: 1/3 having had no exposure to antibiotics, 1/3 having been soaked for 5 minutes in antibiotic solution and 1/3 having been loaded with antibiotic using iontophoresis as per the above technique for 5 minutes with a potential difference of 100 volts. From each specimen a 1mm thick axial section was cut using a diamond saw and this was placed on a nutrient agar plate with a standard *Staphylococcus aureus* preparation (fully sensitive). The plates were then incubated for 24 hours and the zone of inhibition inspected and measured.

The results for the rates of dissolution of the antibiotics from tubular sections of bone 2 cm long, (approx. 5g weight) in 20 ml of saline are shown in fig 9 & 10.

Both antibiotics behaved in a similar fashion, with a logarithmic decrease in their rates of dissolution. There was still antibiotic becoming available up to two weeks after soaking the bone in normal saline solution.

Bioactivity of Antibiotics after Iontophoresis: The results of the nutrient agar plate test of the drugs activity against a fully sensitive *Staphylococcus* are shown in Figures 11a and 11b.

While bacterial colonies can be seen growing up to the bone in the case of bone which has never been exposed to antibiotic there is a zone of inhibition of growth around the bone for those soaked in antibiotic and a larger zone of inhibition around the iontophoresis specimens.

Thermal Effects of Iontophoresis: The temperature plot is shown in Figure 12 for the mean values of 3 specimens. All specimens were initially cooled to 10°C. The plot shows the mean values with the highest and lowest values superimposed. The iontophoretic current was turned off at 15 minutes and recordings of temperature made for a further 3 minutes.

After an initial rapid rise in the first 30 seconds the temperature increases slowly until the current is turned off at 15 minutes. The highest temperature recorded was 35.7°C. This thermal effect is result of the energy dissipation of the 40 mamp current being passed at 100 volts potential difference representing a power generation of 4 watts energy, within the system as a whole.

The results of this work demonstrate that high concentrations of both gentamicin and flucloxacillin can be achieved in sheep tibia after 1 minute and human allograft tibia after 5 minutes of iontophoresis. The procedure is simple and requires no expensive equipment. Further allograft bone may be treated in this way at the time of defrosting the graft prior to implantation in the operating room.

An attempt to simultaneously move gentamicin and flucloxacillin in opposite directions across a cortex has shown that gentamicin moves full thickness, but flucloxacillin penetrates only the periosteal side. This is most likely due to the

precipitation reaction encountered when solutions of gentamicin and flucloxacillin are mixed. The implication in clinical practice is that the gentamicin will penetrate well using this system, but the periosteal surface of the bone will in addition achieve therapeutic levels of flucloxacillin. The preliminary work with methylene blue has shown that this is likely to be the surface that receives the least predictable amount of positively charged ion (in this case, gentamicin.).

While the levels of gentamicin in the bone may appear alarmingly high, if a 500g allograft containing 200mg/Kg were to be implanted, the total dose of drug being implanted is about 100mg, which is well below the usual daily dose of gentamicin on a once daily intravenous regime. The method of calculation of antibiotic concentration in bone has assumed that all antibiotic is washed out of the crushed bone. The levels of antibiotic described above are also considerably higher than levels that have been measured in bone after intravenous administration which vary from 2.4-19.4 mg/Kg and usually reach levels between 0.14 and 0.36 of serum concentration.

It should be understood that the foregoing description of the invention including the principles, preferred embodiments and Examples cited above are illustrative of the invention and should not be regarded as being restrictive on its scope. Variations and modifications may be made to the invention by others without departing from the spirit of that which is described as the invention and it is expressly intended that all such variations and changes which fall within this ambit are embraced thereby. is intended merely to be illustrative thereof.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1.   A bone or bone tissue supplemented with at least a therapeutically useful compound, wherein said compound is concentrated within the bone matrix.

2.   A bone or bone tissue according to claim 1 wherein the therapeutically effective compound is concentrated to an amount between the minimum concentration required for activity of the compound *in vivo* and the maximum concentration that is equal to the safe maximum single dose for systemic administration.

3.   A bone or bone tissue according to claims 1 or 2 wherein the therapeutically effective compounds that can be concentrated into the bone or bone tissue are selected from: antibiotics, antifungal compounds, chemotherapeutic compounds, tissue growth factors, non-steroidal anti-inflammatory agents, such as indomethacin, neuromuscular agents affecting calcium and bone metabolism, anti-viral agents, anti-tuberculosis agents, anthelmintic agents, antiseptic agents, vitamins and minerals.

4.   A bone or bone tissue according to anyone of the preceding claims wherein the therapeutically effective compounds loaded into the bone are compounds that form a salt in solution and ionise to a single positive or negative ion.

5.   A bone or bone tissue according to anyone of the preceding claims wherein the therapeutically effective compound is an antibiotic selected from the following group: flucloxacillin, gentamicin, cephalothin, ticarcillin, ciprofloxacin, nenzl-peniccillin, cefoperazone, cefuroxime, cephazolin and tobramycin

6.   A bone or bone tissue according to claim 5 wherein the antibiotic is either flucloxacillin or gentamicin.

7. A bone or bone tissue according to claim 6 wherein the antibiotic is gentamycin and it is loaded into the bone or bone tissue at a maximum dose of about 200 mg/kg.
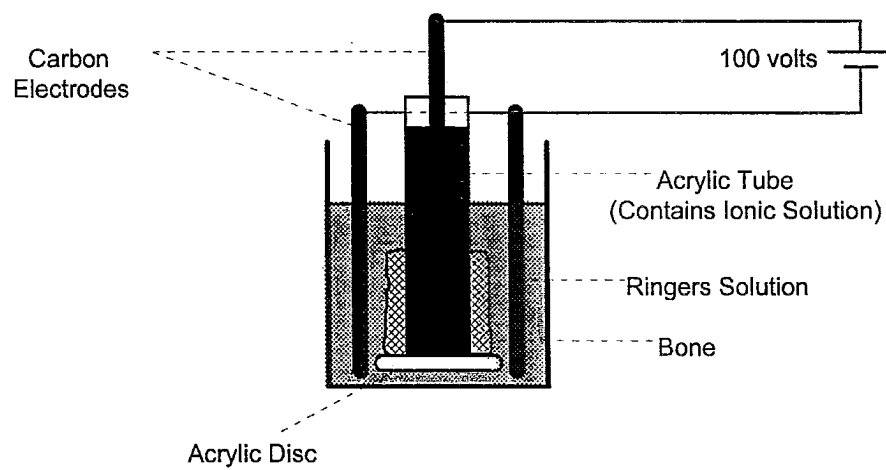
8. A bone or bone tissue according to claim 6 wherein the antibiotic is flucloxacillin and it is loaded into the bone or bone tissue at a while the maximum dose of about 80 mg/kg.

9. A bone or bone tissue according to claim 3 wherein the therapeutically effective compound is an antifungal compound selected from the following group: miconazole, and ketaconazole.

10. A bone or bone tissue according to claim 3 wherein the therapeutically effective compound is a chemotherapeutic compound selected from the following group: 5-fluoro-uracil and vinblastin.

11. A method for supplementing bone or bone tissue with a therapeutically effective compound, wherein said method employs the steps of:

    (i) Exposing bone or bone tissue to a therapeutically effective compound; and

    (ii) Applying a potential difference across said bone or bone tissue such that the therapeutically effective compound is concentrated within the bone or bone tissue.

12. A method according to claim 11 wherein the therapeutically effective compound employed used in the method is concentrated within the bone or bone tissue using an externally applied potential difference.

13. A method according to claim 11 or 12 wherein the therapeutically effective compound is introduced at medically safe levels into tissue surrounding a bone in a patient and an externally applied potential difference is then applied across the bone for sufficient time to concentrate the therapeutically effective compound within the bone.

14. A method according to anyone of claims 11 to 13 wherein the externally applied potential difference is selected such that it is capable of drawing and concentrating the therapeutically effective compound into the bone but does not effect the structural integrity of the surrounding tissue.

15. A method according to anyone of claims 11 to 14 wherein the bone is allograft bone.

16. A method according to any one of claims 11 to 15 wherein the therapeutically effective compounds that can be concentrated into the bone or bone tissue are selected from: antibiotics, antifungal compounds, chemotherapeutic compounds, tissue growth factors, non-steroidal anti-inflammatory agents, such as indomethacin, neuromuscular agents affecting calcium and bone metabolism, anti-viral agents, anti-tuberculosis agents, anthelmintic agents, antiseptic agents, vitamins and minerals.

17. A method according to any one of claims 11 to 16 wherein the therapeutically effective compounds loaded into the bone or bone tissue are compounds that form a salt in solution and ionise to a single positive or negative ion.

18. A method according to claim 16 wherein the therapeutically effective compound is an antibiotic selected from the following group: flucloxacillin, gentamicin, cephalothin, ticarcillin, ciprofloxacin, nenzl-peniccillin, cefoperazone, cefuroxime, cephazolin and tobramycin

19. A method according to claim 18 wherein the antibiotic is either flucloxacillin or gentamicin.

20. A method according to claim 18 wherein the antibiotic is gentamycin and it is loaded into the bone or bone tissue at a maximum dose of about 200 mg/kg.

- 22 -

21. A method according to claim 18 wherein the antibiotic is flucloxacillin and it is loaded into the bone or bone tissue at a while the maximum dose of about 80 mg/kg.

22. A method according to claim 16 wherein the therapeutically effective compound is an antifungal compound selected from the following group: miconazole, and ketaconazole.

23. A method according to claim 16 wherein the therapeutically effective compound is a chemotherapeutic compound selected from the following group: 5-fluoro-uracil and vinblastin.

24. A bone or bone tissue supplemented with at least a therapeutically useful compound, wherein said compound is concentrated within the bone matrix according to the method defined in any one of claims 11 to 23.

Carbon
Anode

Methylene
Blue

Bone disc

Steel Cathode

PD

Figure 1

Methylene Blue

Carbon Anode

Epoxy Cement

PD

Bone

Steel Cathode

Figure 2

Carbon Electrodes

100 volts

Acrylic Tube (Contains Ionic Solution)

Ringers Solution

Bone

Acrylic Disc
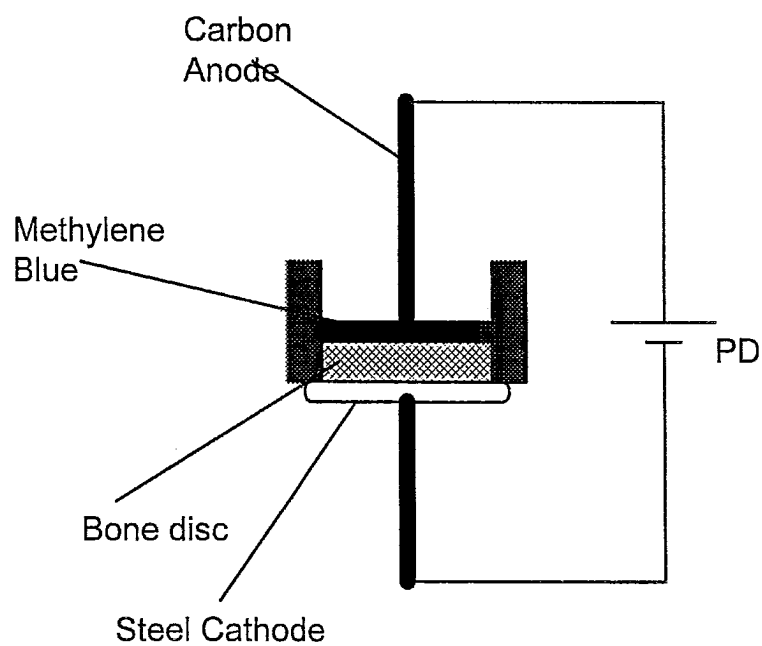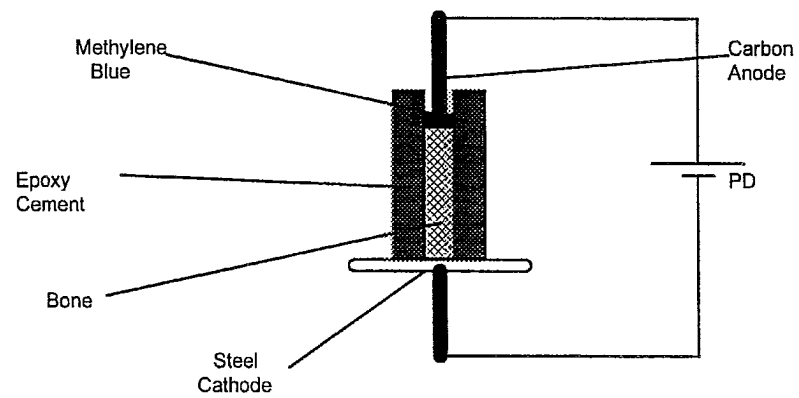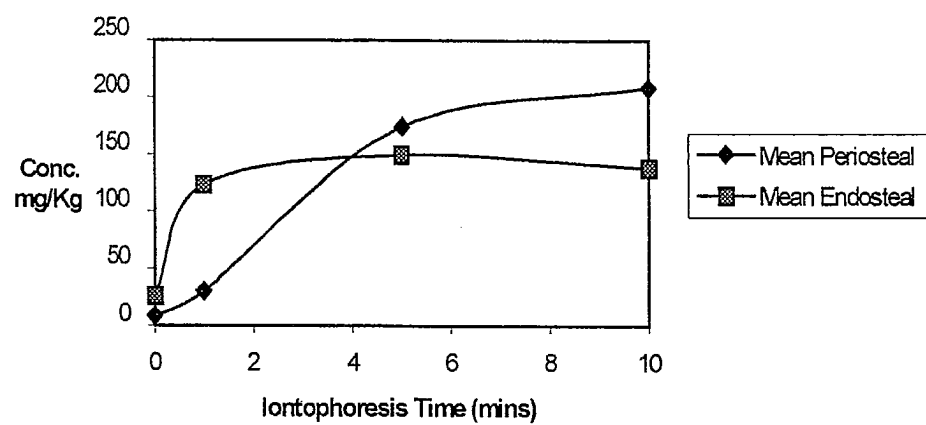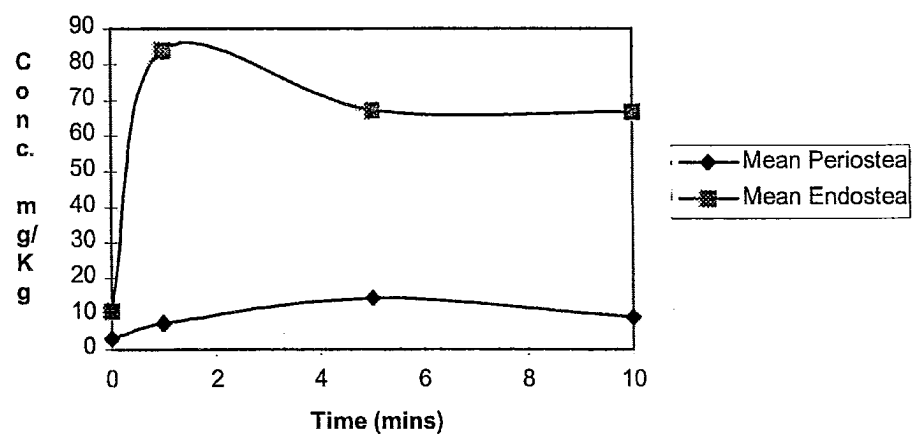
Figure 3

Figure 4

Figure 5

Figure 6

Figure 7

Combined Iontophoresis: Gentamicin & Flucloxacillin
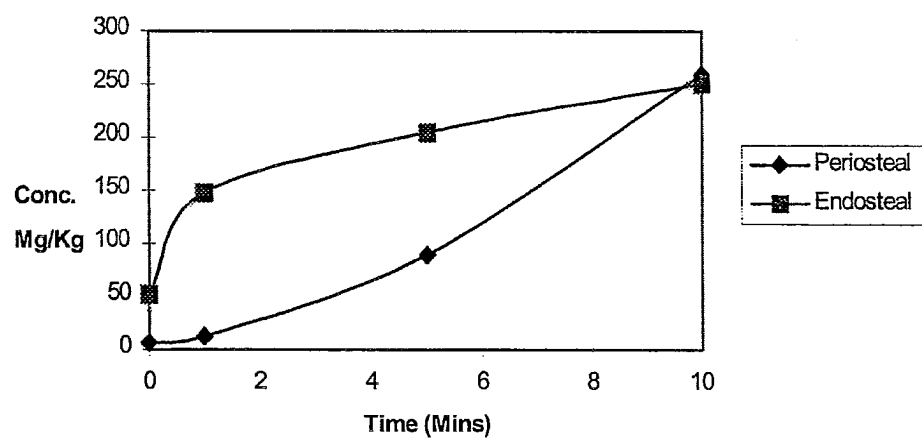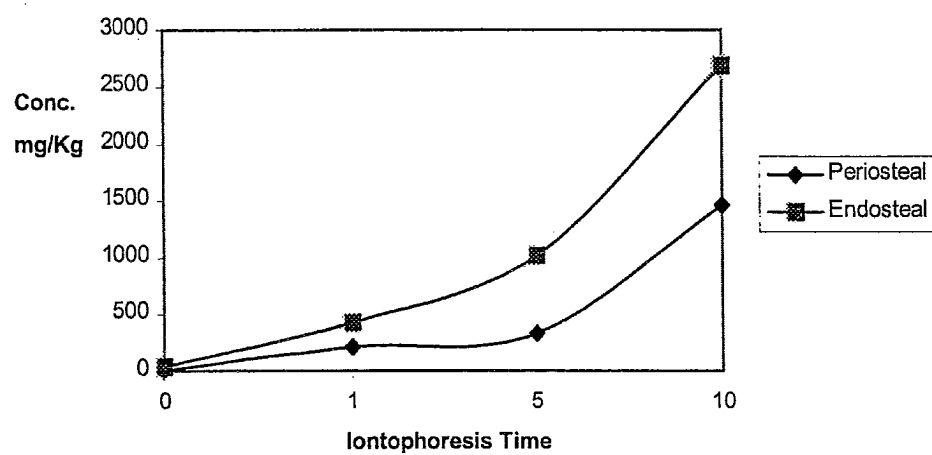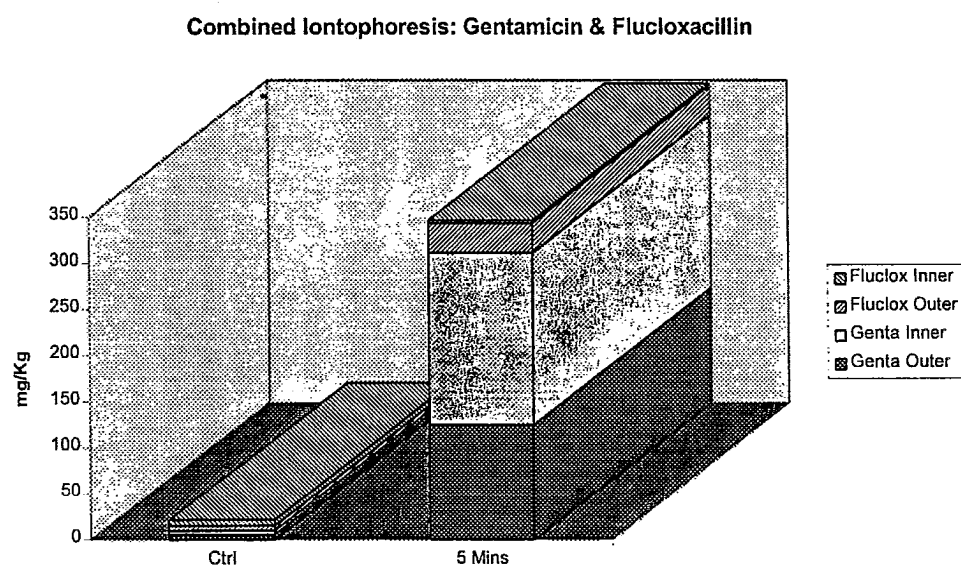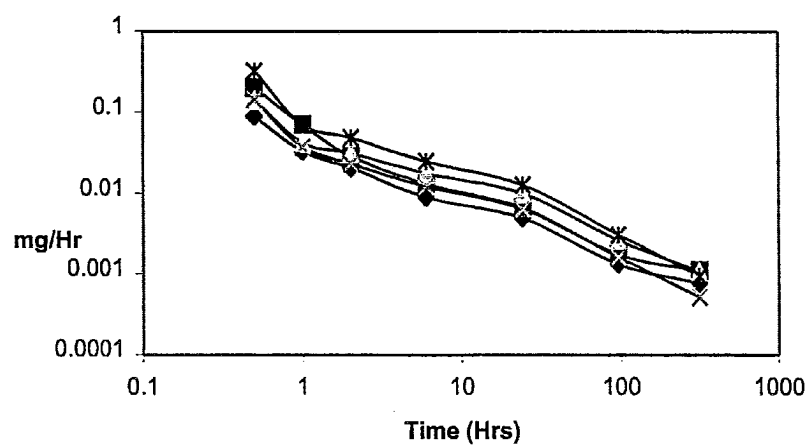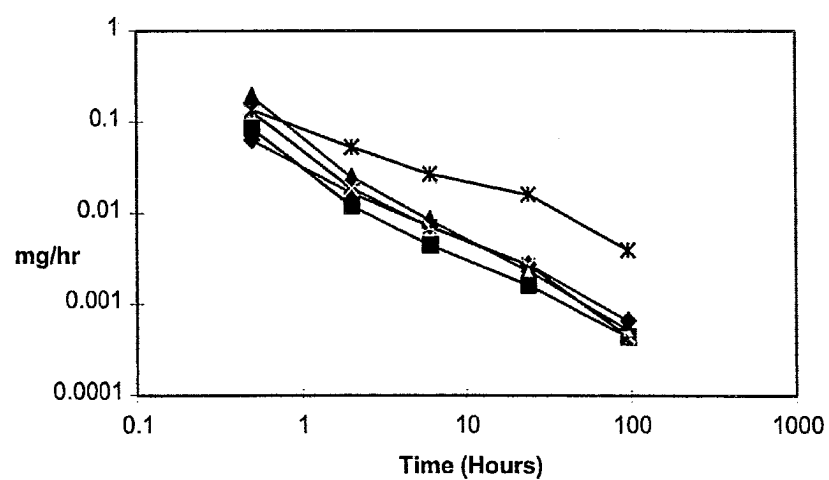
Figure 8
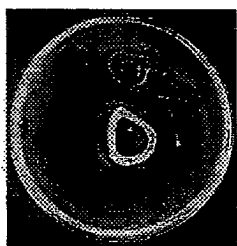
Figure 9
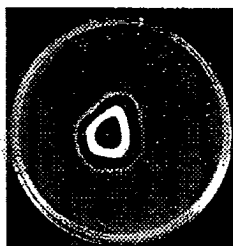
Figure 10
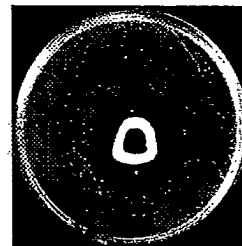
| Control | Soaked 10 mins | Iontophoresis 5 mins |

Figure 11a

| Control | Soaked 10 mins | Iontophoresis 5 mins |

Figure 11b

Figure 12

INTERNATIONAL SEARCH REPORT

A.	CLASSIFICATION OF SUBJECT MATTER

Int Cl^6:   A61K 35/32; C12N 5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B.	FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC: A61K; C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
AU: IPC as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Derwent WPAT: bone or oste or allograft and gentamicin or flucloxacillin

C.	DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	AU 96/47444 (ALG CO) 29 December 1995 C12N 5/00; See Claims 1, 2, 20 and 21, page 15, 29	1-7,11-20
X	AU 96/57945 (SMITH & NEPHEW RICHARDS INC) 19 May 1995 B01F 13/06 21 November 1996 See page 17, lines 14-16	1-5,11-16,24
X	JP 4-279520 (EISAI CO LTD) 31 May 1990 A61K 9/00, A61K 37/34, 5 October 1992	1-3,24

X	Further documents are listed in the continuation of Box C	X	See patent family annex

*   Special categories of cited documents:

"A"   document defining the general state of the art which is not considered to be of particular relevance

"E"   earlier application or patent but published on or after the international filing date

"L"   document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O"   document referring to an oral disclosure, use, exhibition or other means

"P"   document published prior to the international filing date but later than the priority date claimed

"T"   later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X"   document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y"   document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&"   document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 November 1998	1 7 NOV 1998

Name and mailing address of the ISA/AU	Authorized officer
AUSTRALIAN PATENT OFFICE PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No.: (02) 6285 3929	A WILCOX Telephone No.: (02) 6283 2243

INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU 98/00736

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0349256 (OSTEOTECH INC) 3 January 1990	1-7,11-20,24
X	AU 71163/87 (MERCK PATENT GmbH) 18 April 1986, A61L 25/00, 22 October 1987 See entire document	1-7,11-20,24
X	US 4059684 (KULZER & CO GmbH) 22 November 1977	1-7,24

INTERNATIONAL SEARCH REPORT

Information on patent family members

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report				Patent Family Member				
AU	96/47444	WO	97/24434	EP	870012			
AU	96/57945	WO	96/36429	US	5549381			
AU	94/81357	WO	95/13818	EP	9505058	SG	49032	
JP	4-279520	NONE						
EP	349256	US	4994030					
AU	87/71168	CA	1276558	DE	3613213	EP	242674	
		EP	242672	HU	44169	JP	62254764	
		US	4869906	ZA	8702762			
US	4617293	AU	83581/82	CA	1196628	EP	65123	
		ES	512126	ES	8307097	IL	65749	
		JP	58059995	ZA	8203325	DE	3206725	
US	4059684	AU	83581/82	CA	1196628	EP	65123	
		ES	512126	ES	8307097	IL	65749	
		JP	58059995	US	4617293	ZA	8203325	
		DE	3206725	DE	2511122	GB	1511452	

END OF ANNEX